REV CUBANA PLANT MED 2005;10(3-4)

Laboratorio de bioquímica: Escuela de Farmacia. Universidad de Puerto Rico, San Juan, Puerto Rico
Laboratorio de productos naturales: Escuela de Farmacia. Universidad de Puerto Rico, San Juan, Puerto Rico

Inhibition of aldose reductase by herbs extracts and natural substances and their role in prevention of cataracts

Dr. Ángel Guzmán[1] and Dr. Ricardo O. Guerrero[2]

Summary

Cataractogenesis is a common complication that occurs in diabetes mellitus. Aldose reductase is a lens enzyme probably involved in the development of this eye problem. The purpose of this investigation was to screen plant extracts for aldose reductase inhibitors (ARI) and to investigate their possible influence in diabetic cataractogenesis prevention. 13 plants and 3 natural products were randomly selected for our experiment. The 19 extracts originated from plant material which was extracted with ethanol, water and DCM, and assessed for inhibitors of aldose reductase. This enzyme was isolated from bovine lenses homogenates. The enzyme was incubated in a reaction mixture containing 50 mM Na-phosphate buffer (pH 6.2), NADPH, 400 mM $LiSO_4$ and dl-glyceraldehyde. A spectrophotometrical assay was performed in which NADP is produced and its absorption read at 340 nm. *Eugenia borinquensis, Mangifera indica, Eucalyptus deglupta*, and *Syzygium malaccense* were among the best inhibitors. Normal and diabetic Sprague Dawley rats were used to evaluate the *in vivo* effect of *E. deglupta*, *M. indica*, and *E. borinquensis* in cataractogenesis prevention. All of these extracts had preventive effects on the formation of cataracts. It is concluded that further investigation to explain the above findings is necessary. Perhaps the next step will include an activity monitored isolation of the effective principles. Future investigation will ince the isolation of the active principles in these extracts.

***Key words**:* Puerto Rico, plant extracts, aldose reductase inhibition, cataractogenesis prevention.

A cataract is the opacity of the lens that produces painless gradual loss of vision. Acquired cataracts may result from trauma, radiation or metabolic disorders. It may be produced in several conditions such as hyperglycemia, galactosemia, hypocalcaemia and diabetes mellitus.[1] Aldose reductase (AR), the key enzyme of the polyol pathway, is known to play important roles in diabetic complications. The inhibitors of aldose reductase, therefore, would be potential agents in the prevention of one of these complications, namely, the development of cataracts. Through the years, medicinal plants have been the focus of many investigations for the search of aldose reductase inhibitors (ARI). *Ueda* et al.[2] studying the leaves of *Myrciaria dubia* found ellagic acid and two of its derivatives as potent ARI. Three flavonoids isolated from *Brickellia arguta* by *Rosler* et al.,[3] showed anti-cataract activity in rats. Some Chinese herbs have also been investigated as source of ARI with a fluorometric assay.[4] *Kohda* et al.[5] discovered acteoside, an active ARI phenolic glycoside from a 70 % acetone extract of *Monochasma savatierii*. Perillosides A and C were found to be excellent ARI. These monoterpene glycosides were isolated from the leaves of *Perilla frutescens*. The investigators[6] attempted to establish a relationship

between structure and inhibitory activity. Some sulfated flavonoids in *Polygonum hydropiper* were discovered to show potent inhibition against bovine lens aldose reductase.[7] Other dihydroflavonols from *Engelhardtia chrysolensis* were determined to have potent aldose reductase inhibitory activity.[8,9] Other studies showed that flavonoid glycosides,[10] isoflavonoids,[11]and tannoid principles[12] had strong inhibitory activity.

The purpose of this investigation was to screen 13 plant extracts and 3 natural products for aldose reductase inhibitors (ARI) and to investigate their possible influence in diabetic cataractogenesis prevention.

Methods

Plant material

In the present study, the AR inhibitory activity of 19 extracts (see Table 1) was studied along with the *in vivo* effect of three of the best ARI for cataractogenesis prevention. The 19 extracts originated from 13 plant species and three natural products in different solvents. The natural products were acquired in local shops and the plant specimens were collected in the Caribbean National Forest (CNF) by Mr. *Luis Rivera* (taxonomist of the CNF) and in the Botanical Garden of the University of Puerto Rico by Mr. *Julio Figueroa* (US Department of Agriculture) in 2003. Mr. Luis Rivera identified the plants and voucher specimens are maintained in the Herbarium of the School of Pharmacy. Extracts were made by macerating for several days 30-40 g of the ground plant material (leaves or natural product powder) with the solvent, filtering and evaporating the solvent *in vacuo* at 30-40^0 C. Most of the extracts contained flavonoids, according to the magnesium and concentrated hydrochloric reaction.[13] One of the plants, (*E. borinquensis*) is endemic to the cloud forests of the Luquillo mountains, Caribbean National Forest, north-eastern Puerto Rico.

Table 1. *Herbs extracts and natural products evaluated for inhibition of aldose reductase*

Plant	Scientific Name	Family	Solvent	Voucher
1	*Allium cepa*	Alliaceae o Amaryllidaceae	EtOH	PR007
2	*Mangifera indica*	Anacardiaceae	EtOH	PR001
3	*Vaccinium myrtillus* [1]	Ericaceae	H_2O	N/A
4	*Vaccinium myrtillus* [1]	Ericaceae	EtOH	N/A
5	*Nepeta cataria* [2]	Lamiaceae	DCM	N/A
6	*Nepeta cataria* [2]	Lamiaceae	H_20	N/A
7	*Nepeta cataria* [2]	Lamiaceae	EtOH	N/A
8	*Clidemia octona*	Melastomataceae	EtOH	PR153
9	*Calycogonium squamulosum*	Melastomataceae	EtOH	PR008
10	*Psidium guajava*	Myrtaceae	EtOH	PR046
11	*Eugenia borinquenses*	Myrtaceae	EtOH	PR060
12	*Syzygium malaccense*	Myrtaceae	EtOH	PR061

13	*Eucalyptus deglupta*	Myrtaceae	EtOH	PR059
14	*Desmodium adscendens*	Papilionaceae	EtOH	PR002
15	*Coccoloba swartzii*	Polygonaceae	EtOH	PR011
16	*Manilkara bidentata*	Sapotaceae	EtOH	PR029
17	*Daucus carota*	Umbelliferae	EtOH	PR114
18	*Zingiber officinale*	Zingiberaceae	EtOH	PR083
19	*Curcuma longa*[3]	Zingiberaceae	EtOH	N/A

[1] Bilberry (Nature's Herbs®) Modern Products, Inc. Milwaukee, WI 53209, USA,

[2] Catnip Herb (Nature's Way Protects®) Springville, Utah 84663, USA,

[3]Turmeric (Spice Garden®) A Twin lab Division, American Fork, Utah 84003, USA,
EtOH: Ethanol; DCM: Dichloromethane.

Bioassay

The bioassay procedure with some modifications was followed according to *Haraguchi* et al.[7] Bioassay solutions of plant extracts were prepared by dissolving 2 mg of the extract in 50 µL DMSO. After 30 min or more, a 1 mL volume was completed by adding phosphate buffer pH 6.2. Aldose reductase was obtained from bovine eye lenses, kept frozen at -20º C until the day of the experiment. The lenses were removed by lateral incision of the eye, washed with cold distilled water and kept cold. The lenses were deposited in a cold glass tube and the homogenized using a Teflon pestle in 3 volumes of cold 135 mM phosphate buffer (pH 7.0), containing 10 mM β-mercaptoethanol. The homogenate was centrifuged at 13,369 RPM (10 000 × g) for 15 min. The supernatant fluid was placed in a plastic 50 mL conical centrifuge tube and the protein in this fluid was then precipitated, by addition, drop by drop, of a solution 75 % $(NH_4)_2SO_4$. The pellet obtained by centrifugation was dissolved in the same buffer and used as enzyme preparation. This solution containing enzyme was analyzed using the Biuret reaction. Its protein concentration was 4 mg/mL.

The reaction mixture was prepared at 25º C, with a total volume of 1.8 mL, containing 50 mM Na-phosphate buffer (pH 6.2), 0.125 mM NADPH, 400 mM $LiSO_4$, enzyme preparation equivalent to 4 mg protein and 3 mM dl-glyceraldehyde as a substrate with or without plant extract. The reaction was initiated by addition of NADPH and continued by 30 min. Absorbance readings were conducted at 340 nm absorption at the beginning and at the end of the reaction during this incubation time. $NaHCO_3$ 1M was added at the end of the 30 minute incubation period. Quercetin (3.3×10^{-1} to 33.3 mM), a known aldose reductase inhibitor was used as positive control to compare the plant extracts inhibitory activity. A negative control was prepared using 5 % DMSO in phosphate buffer (pH 6.2). The bioassays were run in triplicate and the average inhibitory activities are shown on table 2 as percentages. At the end, the inhibitory activity of the extracts was calculated using the following formula:

$$\% \text{ ARI} = \frac{\Delta \text{ Abs. (Neg. Ctrl.)} - \Delta \text{ Abs. (Extract)}}{\Delta \text{ Abs. (Neg. Ctrl.)}} \times 100$$

Results

Table 2 shows the percentage inhibition values of aldose reductase by the 19 extracts. Among the three evaluated natural products, *C. longa*, known as turmeric (75 %), and the EtOH extract of *V. myrtillus* known as bilberry (67 %), were found to have the best inhibitory activity.

The plant extracts of *Eugenia borinquensis* (82 %), *Eucalyptus deglupta* (88 %), and *Mangifera indica* (92 %) were among the best inhibitors of aldose reductase. Aqueous extracts of each of these plants (30 mg/mL) were assessed for prevention of cataractogenesis in streptozotocin induced diabetic rats (150-200 g) of the Sprague Dawley strain. Groups of ten rats were given plant extract to drink ad libitum for three months while controls received water. Groups were designated as A: rats with *E. Borinquensis*, B: rats with *M. Indica*, C: rats with *E. deglupta*, D: diabetic controls and E: non-diabetic controls. Three months later, significant effects were observed on the rats. It was visually established that all the rats treated with *E. deglupta* extract did not develop cataracts whereas 90 % of those tested with *M. indica* and 50 % of the animals treated with *E. borinquensis* did not develop cataracts, either.

Table 2. *Aldose Reductase Percentage Inhibition of herbs extracts and natural products*

Plant	Scientific Name	Solvent	Aldose Reductase Percent. Inhibition
1	*Allium cepa*	EtOH	1
2	*Mangifera indica*	EtOH	92
3	*Vaccinium myrtillus*	H_2O	32
4	*Vaccinium myrtillus*	EtOH	67
5	*Nepeta cataria*	DCM	18
6	*Nepeta cataria*	H_20	9
7	*Nepeta cataria*	EtOH	21
8	*Clidemia octona*	EtOH	51
9	*Calycogonium squamulosum*	EtOH	0
10	*Psidium guajava*	EtOH	40
11	*Eugenia borinquensis*	EtOH	82
12	*Syzygium malaccense*	EtOH	82
13	*Eucalyptus deglupta*	EtOH	88
14	*Desmodium adscendens*	EtOH	8
15	*Coccoloba swartzii*	EtOH	14
16	*Manilkara bidentata*	EtOH	0
17	*Daucus carota*	EtOH	0
18	*Zingiber officinale*	EtOH	14

19	*Curcuma longa*	EtOH	75

Discussion

The above results indicate presence of bioactive compounds in these three species that prevent cataractogenesis via a possible inhibition of aldose reductase. In relation to these three active plants we have found out that *M. indica* has been studied thoroughly. However, up to date only two investigations have been published in relation to diabetes mellitus. Mangiferin, a xanthone glycoside, isolated from the leaves of *M. indica*, was investigated and demonstrated to possess significant antidiabetic, antihyperlipidemic and antiatherogenic properties.[14] In another study, it was found that the aqueous leaves extract of this plant possesses hypoglycemic activity.[15] No information has been published on the aldose reductase inhibition of this plant. As for *E. borinquensis*, no information (chemical, pharmacological or ethnomedicinal) has been encountered. Nevertheless, it is interesting to note that an investigation on a member of this genus, *Eugenia jambolana* was found to prevent the development of cataracts in alloxan induced diabetic rats.[16] In relation to *E. deglupta*, we have not been able to find, up to date, information concerned with anti-diabetic or anti-cataractogenic activities. It is concluded that further investigation to explain the above findings is necessary. Perhaps the next step will include an activity monitored isolation of the effective principles.

Acknowledgements

The authors are grateful to Mr. *Luis Rivera* of the Caribbean National Forest for his assistance in the identification of many specimens and for the collection of *E. borinquensis*. AG thanks San Juan Bautista School of Medicine and School of Pharamacy, University of Puerto Rico, for the economical support. ROG is indebted to “Proyecto Farmacia” for the assistance in the purchase of reagents and materials.

Resumen

Inhibición de la aldolasa reductasa por extractos de plantas y sustancias naturales y su papel en la prevención de las cataratas

La cataratogénesis es una complicación común que se produce en la diabetes mellitus. La enzima aldosa reductasa está relacionada probablemente con el desarrollo de esta enfermedad. El propósito de esta investigación consistió en determinar si extractos de plantas ejercen una actividad inhibitoria en la enzima y su posible efecto en la prevención de cataratas. Para estos fines, se seleccionaron al azar 13 plantas y 3 productos naturales. Los 19 extractos se originaron del material de las plantas extraídos con etanol, agua y diclorometano. Los extractos fueron analizados por su inhibición de aldosa reductasa. La enzima se aisló de homogenizados de cristalinos de ganado vacuno. Este homogenizado se incubó luego en una mezcla de reacción que contenía amortiguador 50 mM fosfato de sodio (pH 6,2), NADPH, 400 mM $LiSO_4$ y dl-gliceraldehido. Se realizó un análisis espectrofotométrico en el cual se producía NADP, se leyó su absorbancia a 340 nm, y se calculó el porcentaje de inhibición producido por los extractos. Los mejores inhibidores se encontraron en los extractos de *Eugenia borinquensis*, *Mangifera* indica, *Eucalyptus deglupta* y *Syzygium malaccense*. En otro experimento se usaron ratas Sprague Dawley normales y otras a las que se les indujo diabetes para estudiar el efecto *in vivo* de *E. deglupta*, *M. indica* y *E. borinquensis* en la prevención de la formación de cataratas. Todos estos extractos tuvieron efectos preventivos. Futuras investigaciones serán necesarias para aislar los principios activos de estas

plantas responsables de estos efectos.

***Palabras clave**:* Puerto Rico, extractos de plantas, inhibición de aldosa reductasa, prevención de cataratogénesis.

References

1. Gittinger JW. Cecil Textbook of Medicine. 18th ed. Wyngaarden JB, Smith LH, editors. Philadelphia: WB Saunders & Co;1988.p.2289-90.
2. Ueda H, Kuroiwa E, Tachibana Y, Kawanishi K, Ayala F. Moriyasu M. Aldose reductase inhibitors from the leaves of *Myrciaria dubia* (HB &K) McVaaugh. Phytomedicine. 2004;11 (7-8):652-6.
3. Rosler K, Goodwin RS, Mabry T, Varma SD, Norri J. Flavonoids with anti-cataract activity from *Brickellia arguta*. J Nat Prod. 1984;47(2):316-9.
4. Zhang JQ, Zhou YP. Inhibition of aldose reductase from rat lens by some chinese herbs and their components. Pub Med [serial on the Internet]. [cited 2004 Nov 3]. Available from: **http://www.ncbi.nlm.nih.gov**
5. Kohda H, Tanaka S, Yamakoa Y, Yahara S, Nohara T, Tanimoto T, et al. Studies on lens-aldose-reductase inhibitor in medicinal Plants. II. Active constituents of *Monochasma savatierii* Franch. Et Maxim. Chem Pharm Bull (Tokyo).1989; 37(11):3153-4.
6. Fujita T, Ohira K, Miyatake K, Nakana Y, Nakayama M. Inhibitory effects of perillosides A and C, and related monoterpene glycosides on aldose reductase and their structure activity relationships. Chem Pharm Bull. 1995;43(6):920-6.
7. Haraguchi H, Ohmi I, Sakai S, Fukuda A, Toihara Y, Fujimoto T, et al. Effect of *Polygonum hydropiper* sulfated flavonoids on lens aldose reductase and related enzymes. J Nat Prod. 1996;59(4):443-5.
8. Haraguchi H, Ohmi I, Masuda H, Tamura Y, Mizutani K, Tanaka O, et al. Inhibition of aldose reductase by dihydroflavonols in *Engelhardtia chrysolepsis* and effects on other enzymes. Experiencia. 1997; 15;52 (6):564-7.
9. Haraguchi, Ohmi I, Fukuda A, Tamura Y, Mizutani K, Tanaka O, et al. Inhibition of aldose reductase and sorbitol accumulation by astibin and taxifolin dihydroflavonols in *Engelhardtia chrysolepsis*. Biosci Biotechnol Biochem. 1997;61(4):651-4.
10. Haraguchi, Kanada M, Fukuda A, Naruse K, Okamura N, Yagi A. An inhibitor of aldose reductase and sorbitol accumulation from *Anthocepharus chinensis*. Planta Med. 1998; 64(1):68-9.
11. Jung SH, Lee YS, Lim SS, Kim YS, Shin KH. Isoflavonoids from the rhizomes of *Belamcanda chinensis* and their effectson aldose reductase and sorbitol accumulation in streotozotocin induced diabetic rat tissues. Arch Pharm Res. 2002;25(3):306-
12.

Suryanarayana P, Kumar PA, Saraswat M, Petrash JM, Reddy GB. Inhibition of aldose reductase by tannoid principles of *Emblica* officinalis: implications for the prevention of sugar cataract. Mol Vis. 2004;12(10):148-54.

13. Robinson T. The organic constituents of higher plants. North Amherst: Cordus Press;1991.p.205.

14. Muruganandan S, Srinivasan K, Gupta S, Gupta PK, Lal J. Effect of mangiferin on hyperglycemia and atherogenicity in streptozocin diabetic rats. J Ethnopharmacol. 2005; 97(3):497-501.

15. Aderibigbe AO, Emudianughe TS, Lawal BA. Evaluation of the antidiabetic action of *Mangifera indica* in mice. Phytother Res. 2001; 15(5):456-8.

16. Rathi SS, Grover JK, Vikrant V, Biswas NR. Prevention of experimental diabetic cataract by Indian Ayurvedic plant extracts. Phytother Res. 2002; 16(8):774-7.

Recibido: 20 de julio de 2005. Aprobado: 8 de septiembre de 2005.

Dr. *Ángel Guzmán*. Laboratorio de bioquímica: Escuela de Farmacia, Universidad de Puerto Rico, San Juan, Puerto Rico .

[1] **Ph.D. in Biochemistry.**

[2] **Ph.D. in Pharmacognosy.**